Nickel: a new essential trace element[1, 2]

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ABSTRACT

Much indirect evidence exists which indicates that nickel has an essential physiological function. The data have been obtained from pharmacologic, toxicologic, and in vitro biochemical studies. Other evidence has been obtained from chemical analysis of biosubstances which indicates that nickel is consistently present and that nickel concentrations in tissue change in some pathological conditions. Recently, nickel deficiency has been produced in chicks and rats. Chicks raised in a controlled environment free of trace metal contamination and fed a dried skim milk–ground corn diet containing 3–14 ppb nickel exhibited impaired liver metabolism and morphology. This included a reduced ability to oxidize α-glycerophosphate, an increase in the lipid fraction, a decrease in the phospholipid fraction, and an ultrastructural degeneration characterized by dilation of the cisternae of the rough endoplasmic reticulum and by the swelling of the mitochondria. The swelling was in the compartment of the matrix and was associated with fragmentation of the cristae. Dilation of the perinuclear space, condensation of peripheral nuclear chromatin and pyknotic nuclei were also observed. Rats raised under similar conditions also showed abnormalities when deprived of nickel. These included a reduced oxidation of α-glycerophosphate by liver homogenates and abnormalities in the sucrose density gradient polysome profile.—NIELSEN, F. H., AND D. A. OLLERICH. Nickel: a new essential trace element. *Federation Proc.* 33: 1767–1772, 1974.

Examination of the periodic table reveals that of the elements in the transition series from V (23) to Zn (30), nickel is the only one not generally accepted as being essential for animals. This is in spite of the fact that nickel has been suspected of having some physiological role since the 1920's, when it was found to be present in animal tissue. Part of the reason for nickel not being found essential is that nickel-deficient diets have proved difficult to prepare, and appropriate environments for raising animals have not, until recently, been available. Therefore, most evidence presented to date which suggests that nickel has a physiological role has been of the indirect type. The data have been obtained from pharmacologic, toxicologic, and in vitro biochemical studies. Other evidence has been obtained from analyses which indicate that nickel is consistently present in some biological materials, and which show changes in tissue nickel distribution or concentrations in some pathological conditions.

PHARMACOLOGICAL ACTIONS

The earliest animal experiments involving nickel were primarily concerned with its pharmacological effects. In 1926, it was found that parenteral nickel intensifies and prolongs the hypoglycemic effect of insulin in the rabbit and dog (4, 5). Ten years later, it was found that nickel added to posterior pituitary extract prolongs its antidiuretic effect in rats (9, 30). Subsequently, in 1954, it was shown that nickel has an adverse effect on the hypertensive action of epinephrine (20).

Pharmacologic doses of nickel will also apparently alter lipid metabolism. Liver mitochondria from rats fed large amounts of nickel exhibit an in vitro enhancement of cholesterol oxidation (52). In addition, the injection of nickel chloride, or nickel amino acid complexes, into rabbits will increase plasma lipids (11, 12).

The effects of nickel on isolated tissues probably should also be considered pharmacologic. Nickel can substitute for calcium in certain steps of the excitation-contraction coupling of isolated skeletal muscle (13, 14). It is thought that this substitution may occur during the spread of excitation in the sarcolemma and transverse tubular systems. Other investigators have found that nickel can also substitute for calcium in the excitation process of the isolated nerve cell (6, 18). It has been postulated that nickel can substitute for calcium in its binding with a membrane ligand such as the phosphate groups of a phospholipid. Through this substitution, nickel may participate in a manner similar to calcium in the process of nerve transmission and muscle excitation and contraction.

Though none of the aforementioned pharmacological actions are specific for nickel, as other ions can have similar effects, they do show that nickel can act in biological systems. They also suggest that its possible physiological function may involve hormone, membrane and/or lipid metabolism.

TOXIC ACTIONS

Nickel toxicity has been studied in mice (50), chicks (49), and cattle (33). The major findings include depression of the activity of certain enzymes in mice and reduction of nitrogen retention in chicks and dairy calves. Both chicks and calves also exhibited reduced growth. The physiological significance of these studies is unclear.

ALTERATIONS IN BLOOD CONCENTRATIONS OF NICKEL

The concentration of nickel in blood is usually maintained within a characteristic range in man and other species of animals (39). In some pathological conditions, the levels may be altered. For example, serum nickel levels may be

[1] From the American Institute of Nutrition Symposium on *Newer Candidates for Essential Trace Elements* presented at the 57th Annual Meeting of the Federation of American Societies for Experimental Biology, Atlantic City, N.J., April 20, 1973.

[2] Mention of proprietary product does not necessarily imply endorsement by the USDA.

significantly increased in patients with acute myocardial infarction (8, 25, 32, 39, 41, 42) and high concentrations have been found in the serum of patients with acute stroke and acute burns (>25% body surface) (25, 39). Low levels may occur in patients with hepatic cirrhosis and chronic uremia. In contrast to infarction, serum nickel levels are not increased in patients with myocardial ischemia. Acute trauma with fractured bones, acute delirium tremens, and muscular dystrophy apparently do not cause serum nickel to change. Other studies have shown that blood nickel concentrations increase in women with toxemia of pregnancy (23) or with uterine cancer (3). The apparent specificity of the changes in blood nickel concentrations with disease suggests that nickel has some physiological function.

IN VITRO ACTIONS

Nickel is not an indifferent ion in in vitro studies. For example, it has been shown to activate numerous enzymes including arginase (19), tyrosinase (24), deoxyribonuclease (26), acetyl coenzyme A synthetase (51), and phosphoglucomutase (34). However, these studies have not shown nickel to be a specific activator of an enzyme. Another in vitro effect of nickel is the enhancement of the adhesiveness of polymorphonuclear leukocytes (1, 2, 17). Nickel will also stabilize RNA (16) and DNA (10) against thermal denaturation and is extraordinarily effective in the preservation of tobacco mosaic virus RNA infectivity (7, 47). Finally, it has been reported that nickel may have a role in the preservation of the compact structure of ribosomes against thermal denaturation (43–45) and that nickel will restore the sedimentation characteristics of *Escherichia coli* ribosomes which have been subjected to EDTA denaturation.

It is unknown whether these in vitro phenomena relate to an in vivo function of nickel. They do, however, show that nickel can participate in biochemical reactions or systems.

NICKEL CONTENT OF BIOLOGICAL MATERIAL

Significant concentrations of nickel are present in DNA (10, 48) and RNA (38, 47, 48) from phylogenetically diverse sources. It has been suggested that nickel and the other metals which are present may contribute to the stabilization of the structure of nucleic acids. In addition to nucleic acids, it has been found that a metalloprotein in human serum is rich in nickel (21). This protein which is an α-1 macroglobulin and has been isolated from rabbit serum has been designated "nickeloplasmin" (31). It has an estimated molecular weight of 7.0×10^5 and contains 0.9 g atoms of nickel per mole.

These findings suggest that nickel has an apparent structural role in some biosubstances. It is uncertain, however, whether these structural roles are essential for living organisms.

NICKEL IN ANIMAL NUTRITION

The indirect evidence discussed above implies that nickel has a physiological role in living organisms. However, prior to recent research, it had not been conclusively demonstrated that a deficiency of nickel has adverse effects in the intact animal, and thus, its essentiality had not been proved. Direct evidence for the essentiality of nickel will be presented in the remainder of this report.

Nielsen et al. (27–29) found that feeding a diet containing less than 40 ppb nickel resulted in an apparent nickel-deficiency syndrome in chicks. When compared with controls given a supplement of 3–5 ppm nickel, the deficient chicks showed: *a*) pigmentation changes in the shank skin, *b*) thicker legs with slightly swollen hocks, *c*) dermatitis of the shank skin, *d*) a less friable liver which may have been related to the fat content, and *e*) an enhanced accumulation of a tracer dose of ^{63}Ni in liver, bone, and aorta. These findings were observed under conditions which produced suboptimal growth, and the abnormalities in leg structure and shank skin dermatitis were inconsistent.

Sunderman et al. (40) attempted to confirm Nielsen's findings by feeding a diet containing 44 ppb nickel to chicks raised in a slightly different environment. While they found no gross effects, they did observe ultrastructural changes in the liver. These included dilation of the perimitochondrial rough endoplasmic reticulum in 15–20% of the hepatocytes.

To clarify and extend the above observations, improvements were made in the experimental environment used to produce nickel deficiency and a diet was formulated with a nickel content of 3–4 ppb. With this diet and environment it was consistently possible to produce the findings reported herein.

MATERIALS AND METHODS

A major difficulty in the production of nickel deficiency in animals is the preparation of a diet low in nickel. Nickel is ubiquitous. Therefore, the conventional method of diet preparation using purified proteins, or amino acids, carbohydrates, vitamins, and minerals is unsatisfactory. For example, reagent grade minerals are not suitable because some contain as much as 20,000 ppb nickel. To circumvent this problem, the diet must be prepared from natural feedstuffs low in nickel which contain most of the amino acids, vitamins, and minerals essential for the experimental animal.

The diet formulation for the production of nickel deficiency in the chick is presented in Tables 1–3. It was based on dried skim milk, ground corn, and corn oil. The added vitamins included A, D, E, K, niacin, folate, and biotin because skim milk and corn were calculated to provide insufficient amounts. The mineral additions were only 12.8 g/kg of diet. The major portion was $CaCO_3$ (12.5 g) which was relatively low in nickel (approximately 20 ppb). To assure adequate concentrations, Mn, Fe, Zn, Cu, and I

TABLE 1. Composition of basal diet[a]

Ingredient	g/kg Diet
Skimmed milk powder[b]	645.00
Ground corn[b, c]	142.50
Non-nutritive fiber[d]	40.00
Corn oil[b]	100.00
Glycine[e]	5.00
Arginine[e]	25.00
Choline chloride[b]	0.30
Vitamins, fat-soluble[f]	0.11
Vitamin mix (See Table 2)	4.59
Mineral mix (See Table 3)	37.50
	1,000.00

[a] Diet analyzed 3–14 ppb nickel on an air dried basis. [b] Nutritional Biochemicals Corp., Cleveland, Ohio. [c] Corn was acid washed in *experiment 2*. [d] Solka Floc, SW-40, Brown Co., Boston, Mass. [e] General Biochemicals Co., Chagrin Falls, Ohio. [f] The fat-soluble vitamins were: D, L-α-tocopherol acetate (see footnote[b]), 0.01 g and vitamin D_3 (see footnote[e]) (250 IU/drop), 2 drops or approximately 0.1 g. These were added to corn oil prior to mixing it into diet.

TABLE 2. Vitamin mixture for basal diet

Ingredient	g/kg Diet
Niacin[a]	0.0200
Folic acid[a]	0.0012
Menadione[a]	0.0006
Biotin[a]	0.0001
β-Carotene[a]	0.0120
Vitamin A palmitate[b] (250,000 IU/g)	0.0040
Glucose[c]	4.5521
	4.5900

[a] General Biochemicals Co., Chagrin Falls, Ohio. [b] Nutritional Biochemicals Corp., Cleveland, Ohio. [c] Calbiochem Co., Los Angeles, Calif.

TABLE 3. Mineral mixture for basal diet

Ingredient	g/kg Diet
$CaCO_3$[a]	12.5000
$MnSO_4 \cdot 5\ H_2O$[b]	0.2000
Iron sponge[b] (dissolved in HCl[c] before use)	0.0550[d]
$CuSO_4$[a]	0.0150
ZnO[e]	0.0250
KI[b]	0.0005
$(NH_4)_6Mo_7O_{24}$[e, f]	0.0035
Na_2SeO_3[f, g]	0.0006
Ground corn[h]	24.7004
	37.5000

[a] "Reagent" grade, J. T. Baker Chemical Co., Phillipsburg, N.J. [b] "Specpure" grade, Johnson, Matthey Chemicals, Ltd., London, England. [c] "Ultrex" grade, J. T. Baker Chemical Co., Phillipsburg, N.J. [d] In first experiment, 0.0350 g iron sponge was used. [e] "Ultrapure" grade, Alfa Inorganics Co., Beverly, Mass. [f] Added to diets only in experiments in which all dietary ground corn was acid washed (*exp 2*). [g] Alfa Inorganics Co., Beverly, Mass. [h] Nutritional Biochemicals, Corp., Cleveland, Ohio.

TABLE 4. Liver analysis of nickel deficient and supplemented chicks

Group	No. of chicks	O_2 Uptake,[a] μl/hour per mg protein	Total lipid,[b] %	Lipid P,[b] mg/g	Cholesterol,[b] mg/g
		Experiment 1			
Ni def (3 ppb)	12	4.7[c] ± 0.2[d]	6.21[e] ± 0.10		6.29 ± 0.07
+ 3 ppm Ni	12	5.5 ± 0.2	5.78 ± 0.11		6.34 ± 0.04
		Experiment 2			
Ni def (4 ppb)	11	5.4[f] ± 0.2	6.27[e] ± 0.18	1.327[e] ± 0.016	
+ 3 ppm Ni	11	6.0 ± 0.2	5.87 ± 0.05	1.379 ± 0.016	
		Experiment 3			
Ni def (14 ppb)	12	5.5[f] ± 0.1	5.71 ± 0.09	1.318 ± 0.016	5.57 ± 0.07
+ 3 ppm	12	5.9 ± 0.1	5.55 ± 0.10	1.335 ± 0.015	5.67 ± 0.08

[a] Using liver homogenates and with α-glycerophosphate as the substrate. [b] Fresh weight basis. [c] Significantly different ($P < 0.025$) from +3 ppm Ni group. [d] ± SEM. [e] Significantly different ($P < 0.05$) from +3 ppm Ni group. [f] Significantly different ($P < 0.10$) from +3 ppm Ni group.

were added. The basal diet contained 3–14 ppb nickel on an air-dried basis. The variation in nickel content was due to the amount of nickel in individual batches of ground corn. To consistently obtain a diet containing 3–4 ppb, the corn must be acid washed. Because acid washing may remove essential amounts of selenium and molybdenum, they were added to the diet.

In the following experiments, the control chicks were fed the basal diet, supplemented with 3 ppm nickel as $NiCl_2 \cdot 6H_2O$.

A second impediment to the production of nickel deficiency, and also for the production of deficiencies of other trace elements required in very minute amounts, is the environment in which the animals are raised. Significant amounts of trace elements may be present in such sources as caging, feed cups, water bottles, dust in the air, and the skin of the investigator's hands. To prevent such contamination it was necessary to employ techniques developed by Smith and Schwarz (36). The experimental animals were raised in a controlled environmental system[3] in which most, or all, components were made of plastic and the animals were isolated from environmental contamination. Air entering the isolator was passed through a nonmetallic filter to remove dust. The isolators were located in a room maintained at 27 C-29 C, and plastic covered heating pads were used to maintain the temperature at approximately 34 C at one end of each inside cage. When condensation appeared excessive, humidity was decreased by removal of the end caps of the isolators to improve the flow of air out of the isolator.

In the experiments reported here, chicks were used as the initial experimental animal. They often have a higher requirement for specific minerals than other commonly used experimental animals. In addition, they often show more visible gross deficiency signs in studies of mineral requirements. Golden Giant cockerel chicks[4] were distributed at random, six to each inside plastic cage. The diets, as described above, and high purity distilled, deionized water[5] were given ad libitum. Feed cups were not replaced throughout the experiment, but the water cups were replaced every other day. Droppings were removed daily.

The chicks were weighed and examined for abnormalities after being fed their respective diets for 3.5 weeks. They were then exsanguinated by cardiac puncture and decapitated. Portions of fresh liver were taken for measurement of oxygen utilization and electron-microscopic study. The remainder of the liver was frozen at −16 C for subsequent analysis. The oxygen uptake of liver homogenates was measured by the method of Lee and Hsu (22) with α-glycerophosphate as the substrate. Protein was determined by the biuret method after samples (0.1 ml) were dispersed with 0.4 ml of 1% NaCl and 0.5 ml of 10% sodium deoxycholate.

Tissue for electron microscopy was fixed by immersion in a paraformaldehyde-glutaraldehyde mixture buffered at pH 7.4 with sodium cacodylate. Tissue blocks were subsequently post-osmicated in 2% OsO_4 buffered with sodium cacodylate for 1 hour, dehydrated in ethanol, passed through propylene oxide, and embedded in Epon.[6] Thin sections were stained with lead citrate and uranyl acetate.

The lipids were extracted from the liver by the method of Friedman et al. (15). Lipid phosphorus was determined by the method of Taussky and Shorr (46). Liver cholesterol was determined by the method of Searcy and Bergquist (35) on 1 ml of dried extract which had been dissolved in chloroform.

Experiments were also initiated to evaluate the role of nickel in the nutrition of the rat. The diet and conditions used were similar to those used in raising chicks. Slight modification of the diet was made to meet the requirements of the rat (less Ca, Fe, Zn, glycine and choline; more vitamin E). In addition, laminar flow racks[7] were used to hold the plastic rat cages. To bring out the effects in rats, successive generations were raised. Thus, the animals were exposed to deficiency throughout fetal, neonatal, and adult life. Control rats were treated the same except they were fed the basal diet supplemented with 3 ppm nickel. The rats were observed for possible gross abnormalities and impaired reproductive ability. Also, oxygen utilization of liver homogenates of first generation adult rats was examined by the method described previously.

Statistical analysis was performed by the *t* test (37).

RESULTS AND DISCUSSION

After 3.5 weeks, all chicks weighed 350–400 g. Grossly, the appearances of

[3] Germ Free Laboratories, Inc., Miami, Florida. 33100.

[4] Jack Frost Chicks, Inc., St. Cloud, Minn. 56301.

[5] Produced by a "Super Q High Purity Water System," Millipore Corp., Bedford, Mass. 01730.

[6] Epon 812, Warum Chemical, St. Paul, Minn. 55100.

[7] Carworth, Division of Becton, Dickinson and Co., New City, N. Y. 10956.

the deficient and control chicks were similar except for the difference in the pigmentation of their shank skin as described previously (27–29).

In earlier studies (27–29) abnormalities in leg structure and a dermatitis were present in the deficient chicks. In the experiments reported here, the abnormalities were diminished or inconsistent. The decreased incidence or inconsistency of these abnormalities may have been due to modification of the diet and improved environmental conditions. The other gross sign observed in our earlier studies—a decrease in friability of the liver in deficient chicks—was observed in chicks raised under the conditions described above.

Thus, it appears that the change in shank skin color and the effect on liver consistency are related to nickel status but that the other gross signs originally described may be less characteristic of nickel deficiency.

In contrast to the gross signs, abnormalities in biochemical indices of metabolism were more consistently found in the nickel-deficient chicks. These included a decreased oxygen uptake by liver homogenates in the presence of α-glycerophosphate, an increase in liver lipids, a decrease in the liver phospholipids and no apparent change in liver cholesterol (Table 4).

Ultrastructural abnormalities in the hepatocytes (Figs. 1 and 2) were also a consistent finding. These findings were similar to, though more extensive than, those described by Sunderman et al. (40). They included dilation of the cisternae of the rough endoplasmic reticulum and the swelling of the mitochondria. This swelling was in the compartment of the matrix and was associated with fragmentation of the cristae. Other ultrastructural changes included a dilation of the perinuclear space, condensation of peripheral nuclear chromatin and pyknotic nuclei. These ultrastructural abnormalities coupled with the biochemical evidence of deranged metabolism are considered sufficient evidence to indicate that nickel is essential for the chick.

The results of the rat studies are more preliminary in nature because the experiments are still in progress. The results of measurements of the oxidative ability of rat liver homogenates were similar to those found in the chick (Table 5). In addition, sucrose density gradients of liver postmitochondrial supernatants were consistent with a decrease in polysomes and an increase in monosomes in the nickel-deficient rat liver (Fig. 3).

Seven first generation nickel-deficient female rats which were mated had a significant fetal loss at birth (15%) compared with no perinatal mortality in the newborn pups of the six controls.

These preliminary findings suggest that nickel is also essential for the rat.

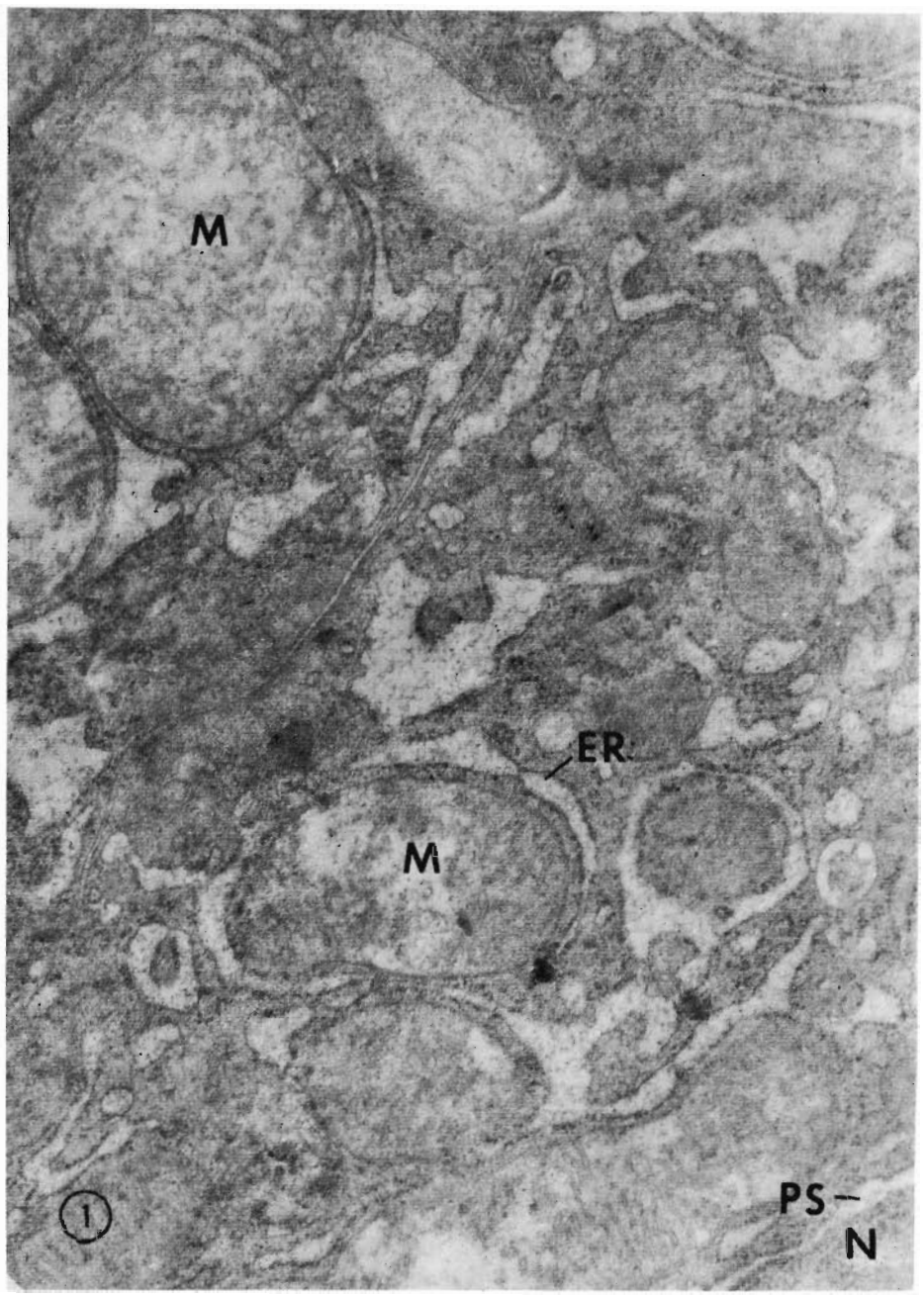
While the above findings indicate that

TABLE 5. Oxygen uptake of rat liver homogenates[a]

Group	No. of rats	O_2 Uptake μl/hr per mg Protein
Ni def (4 ppb)	13	3.20[b] ± 0.08[c]
+ 3 ppm	12	4.17 ± 0.21

[a] α-Glycerophosphate as substrate. [b] Significantly different ($P < 0.001$) from +3 ppm Ni group. [c] ± SEM.

Figure 1. Hepatic cell from a nickel-deficient chick (4 ppb nickel). Swelling of mitochondria (M) was evident in numerous hepatic cells. The swelling was in the compartment of the matrix and appeared to cause fragmentation of cristae. Note also the dilated cisternae of the rough endoplasmic reticulum (ER) and dilated perinuclear space (PS). Nucleus (N). Uranyl acetate and lead citrate. ×30,000.



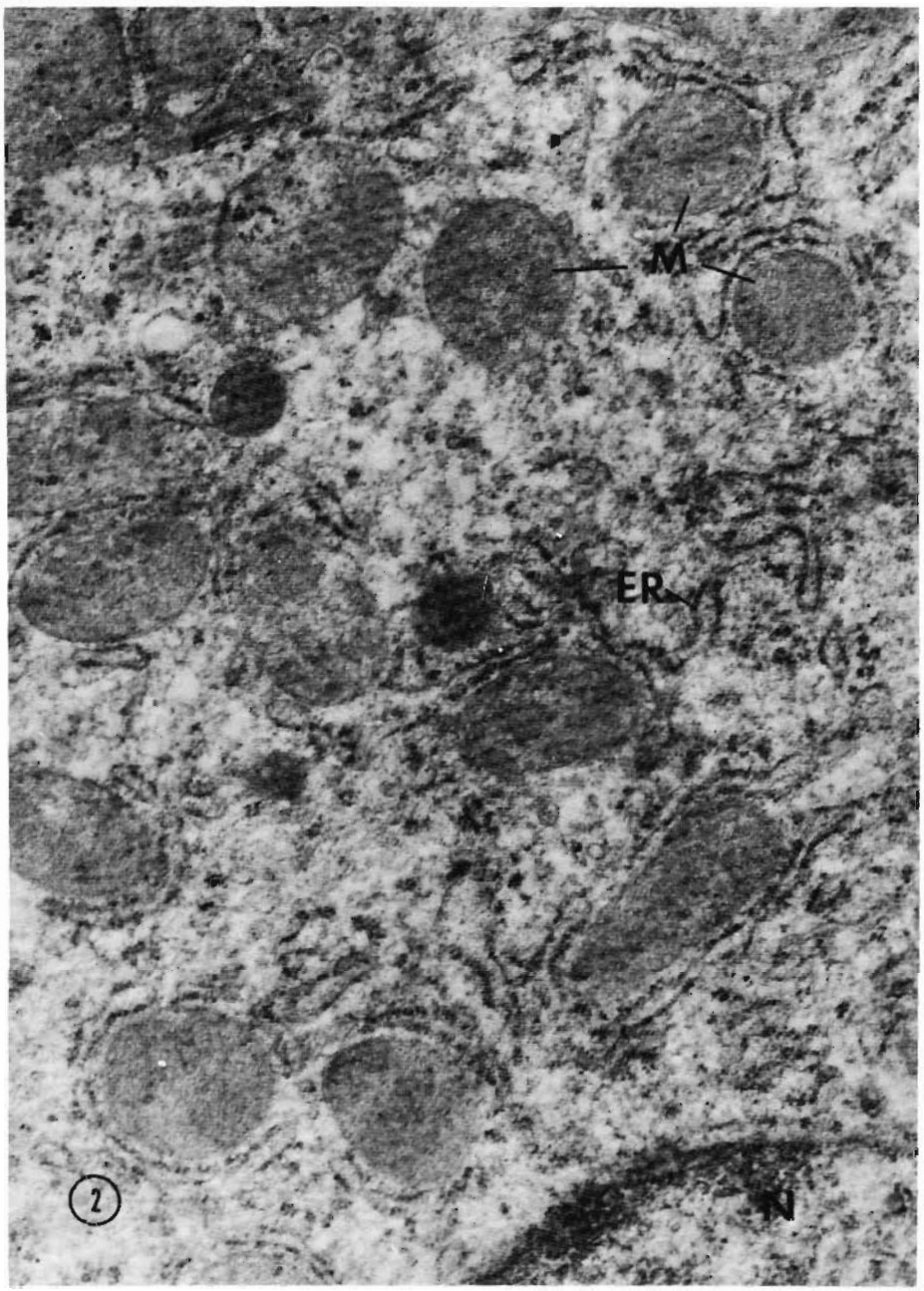


Figure 2. Hepatic cell from nickel-supplemented chicks (3 ppm nickel). Compare mitochondria (M) with those in Fig. 1. Endoplasmic reticulum (ER). Nucleus (N). Uranyl acetate and lead citrate. ×30,000.

nickel is essential, they provide only meager insights as to its metabolic function. An attractive hypothesis is that nickel has a role in the metabolism or structure of membranes. The morphologic abnormalities, impaired oxidation, and changes in phospholipid level in the liver are consistent with this suggestion. In addition, in vitro evidence and our preliminary findings on liver polysomes suggest that nickel may have a structural role in nucleic acids.

SUMMARY

Nickel deficiency in chicks results in suboptimal liver function as evidenced by an ultrastructural degeneration, reduced oxidative ability, increased lipid, and a decreased phospholipid fraction. Rats deprived of nickel also show a reduced oxidative ability in liver and abnormalities in the polysome profile. These findings are consistent with nickel being an essential nutrient for chicks and rats. FP

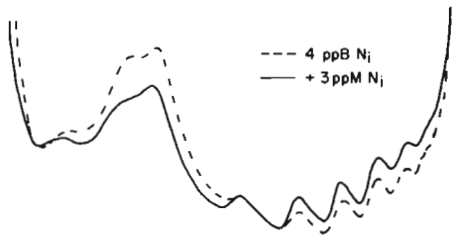


Figure 3. Representative sucrose density gradients of liver postmitochondrial supernatants obtained from 3 nickel-deficient (4 ppb Ni) and 3 supplemented (+3 ppm Ni) rats. Livers were homogenized after being diluted 1:3 (w/v) with medium containing 0.25 M sucrose, 5 mM Tris HCl, 25 mM KCl, and 5 mM $MgCl_2$ at pH 7.4. Supernatants obtained after centrifugation at 4,400 × *g*, at 4 C, were made 0.5% with respect to deoxycholate and centrifuged at 4,687 × *g*, at 4 C. The supernatants were then layered over a linear 15.5–35.5% sucrose density gradient and centrifuged for 160 min at 284,100 × *g* max, at 4 C. The gradients were monitored at 260 nm.

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